THE SPERMATOGENESIS OF SCUTIGERA FORCEPS.

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INTRODUCTION.

In the present paper it is my purpose to note briefly some observations upon the spermatogenesis of *Scutigera forceps*. The spermatogenesis of *Chilopoda* has been the subject of a number of investigations during the last few years besides the earlier ones of Carnoy and Prenant. Carnoy, in his pioneer monograph on "Cytodiérèse Chez les Arthropodes," demonstrated the value of this material by a series of observations on *Geophilus*, *Lithobius*, *Scutigera* and *Scolopendra*, although many of his results have been disproved by later investigators. The most suggestive part of this work has to do with the various kinds of nucleoli and their composition and structure. He divided these bodies into four classes: *nucléoles nucléiniens*, *nucléoles plasmatiques*, *nucléoles mixtes* and *nucléoles noyaux*. In Chilopods, he found two of these: nucléoles noyaux in *Lithobius forficatus*, *Scutigera arachnoides* and *Geophilus;* and nucléoles plasmatiques in *Scolopendra dalmatica*.

Prenant worked upon *Scolopendra* and *Lithobius*, his results being very similar to those obtained later by P. and M. Bouin. These two, in connection with R. Collin, have worked upon several forms of Chilopods, *Lithobius forficatus*, *Geophilus linearis* and *Scolopendra morsitans*. These authors have concerned themselves principally with cytoplasmic structures, although they have given some attention to nuclear structures as well. The large nucleolar bodies found in *Lithobius* and *Geophilus*, according to them, are of an achromatic nature, and would correspond more closely to Carnoy's nucléole plasmatique. The chromosomes are not derived from this body, but arise from granules scattered through the nucleus.

The results obtained by Meves and von Korff upon *Lithobius forficatus* are, in general, in accordance with those of Bouin. They take the same view in respect to the origin of the chro-

mosomes and regard in the same way the composition of the nucleoles.

Blackman ('01, '03) on the other hand, confined his work on *Scolopendra heros* principally to nuclear structures. According to him the large nucleolus found during the resting stage is almost entirely chromatic in nature, and from this body the chromosomes are directly derived at the opening of the active prophase. This structure, which he called the "karyosphere," would correspond to Carnoy's nucléole nucléinien.

Technique.

The animal from which my material was obtained is the black-banded Centipede, or wall-sweep—*Scutigera forceps*. The specimens were collected during the spring and early summer. The animals were killed and their testes immediately removed and placed in Gilson's aceto-nitric sublimate, where they were left for a length of time ranging from twenty-four to forty-eight hours. They were then removed, washed in running water for several hours, run through ascending grades of alcohol from 30 per cent. to 70 per cent., where they were treated with iodine solution to remove the crystals of corrosive sublimate, and preserved in fresh 70 per cent. alcohol. The material was embedded by the usual method in paraffin; sections varying in thickness from three to six micra were cut, fixed to the slide with albumen water and stained by one of the following methods:

The staining method productive of the most satisfactory results is Heidenhain's iron-hæmatoxylin. Sections were stained for observing different structures by varying the degree of extraction of the color. For some purposes, however, the sections stained with iron-hæmatoxylin and counter-stained with congo-red were more satisfactory. For the purpose of a micro-chemical test, Flemming's three-color method may be employed to great advantage. When stained in this manner, the different parts of the cell take on the following colors: the net-work stains a grayish purple, the centrosomes red, the chromatin in the dividing stages and in the karyosphere stains a transparent red, and in the diffuse condition found in the prophases and late telophase stains purple. The achromatic nature of the neucleoli is shown by the fact that

they stain scarcely more densely than the linin and assume a similar grayish appearance.

Observations.

Early in the spermatogonial stages, cells may be seen aggregated in separate groups which are surrounded by definite membranes, or cyst-walls (Fig. 3). The number of cells in these groups varies considerably — in those counted, from eight to over a hundred. This may be due to either or both of two causes. According to the general conception of a cyst, it is an aggregation of cells consisting of all the descendants of one primary spermatogonium. Observations supporting this theory have been afforded by St. George, '76 (*Rana temporaria*), Henking, '91 (*Pyrrhocoris apterus*), Toyama, '94 (*Bombyx*), Montgomery, '98 (*Pentatoma*), Paulmier, '99 (*Anasa*), McGregor, '99 (*Amphiuma*), and Sutton, '00 (*Brachystola*). However, from observations upon later stages, it is evident that in *Scutigera* these cysts are not such permanent structures. By a series of careful counts taken during the spermatogonial and early spermatocytic stages, it has been found that the number of cells in cysts in the same stage of development exhibits considerable variation. The counts of early spermatocytes shows them to exist in groups of from twenty-eight to forty. In the spermatogonia the results were somewhat different. In these, the highest number of cells found in one cyst was one hundred and eight, while the average number in cysts supposed to contain cells in the last spermatogonial division was about sixty. This, to my mind, can be due to but one cause : The cysts are not definite structures, but either by fusion cysts containing a larger number of cells are formed, or, by the division of one, two cysts containing a reduced number are produced. Sutton, in his paper on *Brachystola*, has estimated that the number of cells in one cyst at the close of the division period is two hundred and fifty six. In this case, all of the descendants of one primary spermatogonium are contained within one cyst, but in *Scutigera*, either this is not the case, or the descendants of one of these primary spermatogonia are much fewer in number. That the former is true rather than the latter, is indicated by the fact that in those cysts con-

taining the largest number of cells, all are in practically the same stage of development, and is further supported by the fact that, almost without exception, where cysts contain the smaller numbers, several composed of cells in practically the same stage of development are to be found in the same region of the testis.

The spermatogonia are small spherical cells, averaging about eleven or twelve micra in diameter. During the late prophase of the last spermatogonial division, the chromosomes lie in the clear nuclear area at the center of the cell (Fig. 1). These chromosomes, with the exception of one which stains more darkly than the others, present a dense granular appearance when stained with Heidenhain's iron-hæmatoxylin. The total number of these chromosomes is constantly thirty-seven (Fig. 1), which, if the accessory chromosome is excluded in both cases, is twice the number found in the spermatocytes, showing that this element remains distinct throughout the whole period of spermatogenesis.

During the metaphase of the last spermatogonial division the chromosomes may be seen lying in the equatorial plate, so placed as to form a ring; but owing to the minute size of the component elements and their close proximity to one another, they appear rather as a solid mass than as separate chromosomes (Fig. 2). When viewed in cross section, they present the appearance either of a plate densely crowded on the outside, with separate chromosomes scattered throughout the middle, or of a ring. The centrosomes may be seen lying in the cytoplasm at some distance from the cell-wall and at this period appear as minute particles staining an intense black with the iron-hæmatoxylin. Emerging from these points and directed towards the cell-wall are astral rays which are so fine and delicate as to appear mere elongations in the reticulum of the cytoplasm, rather than definite structures. The spindle-fibers, however, stain intensely and may be seen as separate threads connecting the chromosomes with the centrosomes.

In the early telophase, after the two groups of chromosomes have moved to opposite ends of the cell, the spindle threads, still staining intensely, lie stretched between these two masses, while the centrosomes have become lost (apparently) among the chro-

mosomes and the astral rays have disappeared. As the telophase advances, the chromosomes become more granular, stain less deeply, and appear in the next stage (Fig. 3), as a mass of dense granular threads. Already the growth period has begun, and a slight increase in the size of the cell may be observed. At this period one other important feature may be mentioned. This has to do with that peculiar structure, first clearly recognized by McClung ('99) as a chromosome, and described by him under the name of accessory chromosome. This element may be distinguished from the other chromosomes at this period by certain peculiarities in its form and behavior. During the telophase, while the other chromosomes have been lengthening into diffuse, flaky segments, this structure has remained unaltered and still retains the stain with the tenacity of a chromosome during the metaphase. As is to be expected from its composition, its reaction to stains is quite different from that of the ordinary chromosomes. When stained by Flemming's three-color-method, this element takes the saffranin, whereas the others retain the gentian violet.

During the period immediately following, the cell gradually increases in size, and the reconstruction of the nuclear membrane takes place. By this time (Fig. 4), the cell has increased about one half and the nucleus is considerably larger in proportion than at any other period. The cytoplasm, moreover, stains more lightly and shows scarcely any structure. The chromosomes, immediately after assuming their thread-like shape, lie tangled together in a close heavy mass (Fig. 3), but by the time the nuclear membrane has formed, they begin to spread out through this vesicle and the separate threads may be distinguished (Fig. 4). While this is taking place, these threads of chromatin have gradually become more granular and diffuse, and very evidently fewer in number, while the small black body, above pointed out as the accessory chromosome, has apparently become larger. Into the composite structure, hereafter to be called the karyosphere (Blackman, '03), the diffuse masses of chromatin gradually accumulate (Figs. 5–6) until the nucleus outside of the karyosphere is entirely free of chromatin, and only the linin net-work is to be seen.

Another noteworthy feature characteristic of this period is the presence of metaplasm in variable amounts within the nucleus. Immediately after the reconstruction of the nuclear membrane, when the dense mass of chromatin is beginning to become more diffuse, so that the presence of foreign matter could easily be detected, the nucleus never contains more than the one small round body, the accessory chromosome, beside the mass of thread-like chromosomes. But as the cell increases in size, others gradually appear in the nucleus, and less frequently in the cytoplasm. These bodies are homogeneous, and, like the karyosphere, take a dense black stain with the iron hæmatoxylin. In number they vary widely, some cells being apparently entirely free of them, others containing them in large numbers; while in size they range from scarcely distinguishable particles to large spheres. These bodies seem to be largest and most numerous during the mid-growth period. As the prophase approaches, they seem to decrease in size, and by the time the karyosphere breaks up, their remnants appear as mere particles scattered through the nucleus. Those found in the cytoplasm persist about the same length of time, although there they are much less common than in the nucleus. These bodies present in both nucleus and cytoplasm, correspond to the "ergastoplasmiques" of P. and M. Bouin, which are said to arise through the breaking down of the spindle fibers and which disappear at the opening of the prophase. Meves and von Korff found similar structures in *Lithobius*, but these are often present throughout all the stages of mitosis.

While these changes have been in progress, the cell has gradually been enlarging and by the time of the opening of the active prophase, it is many times its original dimensions. Fig. 7, which represents a cell of about average size, has an approximate diameter of seventy-five micra, while occasionally one may attain the enormous size of one-hundred micra.

Throughout this period, the karyosphere has maintained about the same appearance, excepting its increase in size. During the earlier stages, at the time when the chromatin was accumulating, it appeared to be sharply granular, and to take the stain more densely in some regions than in others; but as the growth period progresses, the karyosphere, in more darkly stained sections, shows

no differentiation of structure (Fig. 7). However, in sections two or three micra thick, from which the color has been sufficiently extracted, a careful study of the karyosphere under a high magnification, reveals the fact that it is not a homogeneous body, but is a complex mass, various parts of which react differently to the stain (Fig. 8). When colored with the iron hæmatoxylin, it appears to be composed of dark irregular masses, and between them smaller areas which stain brown and appear to be of a homogeneous consistency (Fig. 8, *b*, *c*, *d*). On the other hand, the larger masses are of slightly granular nature and commonly appear black. When the stain has been sufficiently extracted, however, they become grayish, and this color argues their chromatic nature, just as the brownish color without testifies to the achromatic nature of the karyosphere.

Evidences of vacuoles within the karyosphere may likewise be seen (Fig. 8, *b*, *c*, *d*) in thin sections lightly stained in the same manner, but their presence is shown much more clearly when in addition to the iron-hæmatoxylin congo-red is used (Fig. 8, *b*, *e*). These vacuoles in varying numbers may lie irregularly throughout the substance of the karyosphere.

From the growth period, the cell passes immediately into the active prophase. The first indication of this stage has to do with the appearance within the nucleus of the chromatic segments. As in *Scolopendra heros* (Blackman, '01), the first sign of activity is a very slight change in the structure of the karyosphere (Fig. 8, *e*). The texture of this body becomes more loose and the distinction between chromatin and achromatin becomes more marked (Fig. 8, *e*). This, however, is so slight that it would probably pass unnoticed were it not for the more striking phenomena which now follow. That the darker portions noted as occurring in the karyosphere at an earlier stage are chromosomes, appears very probable from the fact that they now seem to break off separately from its mass and to be directly transformed into short, thick, granular threads (Fig. 10).

It would naturally be supposed that, as the chromosomes leave, the karyosphere would become smaller, but such is not the case; even after a number of chromosomes are to be seen lying in the nuclear area the karyosphere is apparently as

large as before (Fig. 8, *g*, *h*, *i*, *j*). On close inspection, however, it may be seen that the vacuoles have greatly increased in size. Often these become united, and form one large vacuole at the center of the karyosphere, around which lie the remaining masses of chromatin (Fig. 8, *g*, *h*, *i*). When finally all these have emerged, there is left a mass of material staining more or less darkly, probably a true nucleolar portion of the karyosphere, and one small, round, dense black body, the accessory chromosome (Fig. 8, *i*). This latter presents exactly the same appearance as when last seen during the telophase, except for a slight increase in size, which may readily be referred to natural growth. The remaining mass, or true nucleolar portion of the karyosphere (fig. 8, *j*), now breaks up into small round bodies of more or less irregular size, which soon become indistinguishable from the remains of the metaplasm still lying in the nucleus during the growth period (Fig. 12, 13, 14). For this reason it is impossible to determine the ultimate fate of these bodies; for all seem to be of about the same size and appear to take the stain equally well.

Immediately after leaving the karyosphere, the chromosomes, as was shown above, shorten into dense granular cords (Fig. 9). These now undergo a longitudinal cleavage (Fig. 10), and the double thread of chromatin breaks up into a number of short portions of irregular size (Fig. 11). Whenever the position of these made counting possible, the uniform number of six in each half was to be observed.

Until the opening of the active prophase, the nucleus appears very clear, much more so than the cytoplasm, and contains a finely granular and irregular linin net-work, the meshes of which are much coarser than those of the cytoplasm. Metaplasm bodies left over from the preceding growth period may occasionally be seen caught in the meshes of this net-work, but with the exception of these and the karyosphere, no other structures are visible within the nucleus. Just preceding the emerging of the chromosomes from the karyosphere, however, the nuclear area becomes clouded over here and there with very thin, diffuse masses in which the segments of chromatin seems to become entangled as they pass out into the nucleus. It is these masses of achromatic matter which Bouin (*Lithobius forficatus*), Bouin an l

Collin, '01 (*Geophilus linearis*), Meves and von Korff, '01 (*Lithobius forficatus*) probably mistook for chromatin. In addition to the fact that the chromosomes may actually be seen emerging from the karyosphere, the achromatic nature of this substance is clearly shown by its staining reaction.

At first the chromosomes appear to lie loosely in these achromatic masses (Fig. 8), but as the threads of chromatin split, becoming shorter and thicker, the enveloping substance likewise contracts, staining constantly a darker brown, until when the thread breaks into the smaller fragments (Fig. 11) this envelops them and appears to hold them together. Soon these balls of chromatin become massed more closely together in more or less irregular shapes (Fig. 11, 12), but they always assume as a general outline, the typical form of the tetrad (Fig. 12). This particular shape is often obscured by the unequal sizes of the chromatin fragments, and the distortion of the enveloping sheath which, now being less diffuse, takes a considerably darker stain (Fig. 13). But in all cases favorable for observation, *i. e.*, such instances as when the whole body appears lying flat in one plane, the general outline of the tetrad is plainly discernible.

Thus it seen that while the tetrads in *Scutigera* present characteristics peculiar to themselves, still they are of the same general type found in other arthropods. The only detailed description of tetrad formation in Myriapods has been furnished by Blackman ('03), in *Scolopendra*. According to his description this occurs through a longitudinal and a transverse cleavage in the chromosome segments. The longitudinal division occurs first, and is followed by a bending of the two halves of the segments at their centers, giving the first indications of the second cleavage. "The short processes thus produced elongate at the expense of the quadripartite segment until a cruciform figure is produced, the four arms of which are of about equal length." In *Scutigera*, this process is naturally much obscured by the breaking up of the chromosome segments into the unequal fragments; but when these have again become united by the contraction of the segment, the resulting figure closely resembles that in a similar stage in *Scolopendra*.

As the chromosomes continue to contract, the smaller glob-

ules composing them become fused closely together, and from now on the chromosome always appears as a single structure (Fig. 13 *et seq.*). The enveloping sheath likewise continues to draw more closely about it, until it is finally indistinguishable from its contents (Fig. 15). By the time the last chromosome emerges from the karyosphere, those issuing first have already begun to undergo this operation; but, since each assumes the tetrad form very quickly after making its appearance in the nuclear area, presently all seem to be in an equally advanced stage of development (Figs. 12, 13, 15).

The history of the centrosome is one of the most difficult questions in the study of spermatogenesis of *Scutigera forceps*, on account of the peculiar nature of the cytoplasm. In structure this consists of an exceedingly fine and regular net-work, staining brownish with the iron-hæmatoxylin. A further difficulty is due to the presence in the cytoplasm of small bodies, probably of the same nature as the larger bodies mentioned above. These particles are indistinguishable from the centrosomes, except when the latter may be indicated with certainty by the surrounding astral rays; but these rays offer no sure solution of the problem, for they are of an extremely delicate nature, and endure for only a brief period. Carnoy encountered the same difficulty in *Scutigera arachnoides*, of which he says: "Les figures caryocinétiques y sont plus déliées et moins demonstratives" than in *Lithobius forficatus*, which he has already described as being hard to study on account of its having such dense and opaque cytoplasm.

Because of these difficulties, the centrosomes cannot be distinguished with certainty until the opening of mitotic activity. In the early prophase (Fig. 12), a pair of minute granules may be observed, situated in the cytoplasm at each side of the nucleus, about one third of the distance between its membrane and the cell-wall. These lie in a transparent clear space from which astral rays of exceeding delicacy extend out for a short distance into the cytoplasm, where they gradually become lost in the net-work. During the breaking down of the karyosphere and the formation of tetrads, these centrosomes remain stationary in the cytoplasm, and the asters maintain a nearly uniform size and strength.

The changes in the achromatic part of the nucleus during the progress of the prophases are as considerable as those in the chromatin. During the growth period the contents of the nucleus, consist of the karyosphere, metaplasm granules and a coarse linin net-work. The interspaces of this net-work are large and clear, contrasting decidedly with the thick yellowish appearance of the cytoplasm (Fig. 7). The appearing within the nucleus of the cloud-like achromatic substance, which later forms envelopes for the chromosomes, has already been described (Fig. 9). During this process, the net-work may be seen unbroken between these darker masses; but soon after the chromosomes commence to form, the threads of this net-work begin to coalesce and the meshes gradually become larger and more irregular (Figs. 10, 11, 12).

Thus we have the general appearance of the cell at about the mid-prophase (Fig. 12). The nucleus occupies the central position, while on each side, well out from its membrane, lies a centrosome. Each of these consists of two dark granules situated close together and surrounded by a very delicate aster. Within the nucleus the threads of the net-work have coalesced considerably and now form large and very irregular meshes. Several small, dark masses, either metaplasm or nucleolar part of the karyosphere, may be seen at various places in the nucleus. The chromosomes have split longitudinally, broken into small round unequal segments which have drawn together, and assumed the typical form of the tetrad. Around these, the achromatic masses which serve as envelopes, have contracted until no longer discernible.

The late prophase, judging from the comparatively few cells to be found in this stage, endures but a short period. The chromosomes continue to contract and to increase in density and staining ability until they are reduced to but a small fraction of their former size (Fig. 14, 15) when they lose their granular nature and stain an even, intense black. Immediately after continued contraction, they lose also the tetrad form (Fig. 16), and assume the shape of the "diplosome" described by Bouin (*Lithobius forficatus* and *Geophilus lineatus*). At the breaking down of the karyosphere, the accessory chromosome follows the

others into the nuclear area. At first it may be distinguished from them by its small, regular form and intense stain. But when the others have contracted into the dumb-bell shape, they are of uniform size with the accessory, stain similarly and thus are indistinguishable from it. But that this element assumes its position also in the equatorial area at the time of the metaphase is shown by the fact that there are nineteen chromosomes now present whereas but eighteen chromatin segments could be seen emerging from the karyosphere, and but eighteen tetrads observed later.

As the cell approaches the metaphase, the karyoplasm continues to break down, and assumes more and more the appearance of separate threads. At first these are wavy and irregular and often much branched, being, in fact, but segments of the linin reticulum. Later, however, at the time of the disappearance of the nuclear membrane, they straighten and lie like single threads in the cytoplasm. The centrosomes, meanwhile, have remained in the cytoplasm at some distance from the nuclear membrane. As this fades away, the threads formed from the network of the nucleus stretch from one centrosome to the other, and, losing their granular consistency, take on the form of distinct, definite fibers.

Preceding the breaking down of the nuclear membrane, the nucleus often wanders from its central position in the cell and assumes a position near the cell-wall. Bouin ('01), in *Geophilus linearis*, describes two methods of cell-division; one "division axiale," which represents the usual type where the centrosomes lie at opposite poles of the cell with the spindle between; the other, "division laterale," in which the spindle lies near the periphery and is often tangent to the cell-wall. In the latter case, he says: "Les corpuscules centraux et les sphères s'attachent contre la face interne de la membrane cellulaire, la ligne que les réunit est souvent d'une longueur moindre que le grand diametre de la cellule . . . Les extrémités du fuseau peuvent se trouver si voisine des centres cinétiques qu'elles paraissent se continuer avec la substance de ces formations."

But although cells answering to this description are often to be found in *Scutigera* before the metaphase, there does not seem to

be two methods of division; for the more nearly the cell approaches the metaphase, the more nearly the spindle extends through its central axis.

The fibers of the spindle, when first formed, are short and the ends converge to a point in the centrosomes, which, at this period, do not lie upon the cell-wall, but retain their position in the cytoplasm (Fig. 16). However, the centrosomes gradually move apart and come to lie on the cell wall at opposite sides of the cell. Meanwhile, the chromosomes have taken up their position in the equatorial plate and the spindle fibers extend directly through the center of the cell. They do not now, however, extend from centrosome to centrosome, but lie free in the cytoplasm with ends toward these bodies. Their extremities, while converging somewhat, do not meet in a point. Blackman, '01 (*Scolopendra heros*), described a condition closely resembling this during the formation of the spindle. The centrosomes, however, are upon the nuclear membrane at the time it begins to disappear, but the formation of the spindle and its appearance in early stages closely resembles that found in *Scutigera*. As the centrosomes recede toward the cell-wall, the spindle fibers remain united at the same point (the "apical point") from which center of convergence parallel linin strands extend to the centrosome, while from around these latter emerge the astral rays. Meves and von Korff, '01 (*Lithobius forficatus*), describe conditions more closely resembling those found in *Scutigera* after the formation of the spindle is established, although during this process, the similarity is not so close. Before the disappearance of the nuclear membrane, the centrosomes, surrounded by the centrosphere, have already taken their place upon the cell-wall. During the metaphase, he describes them thus: "Die Spindelfasern liegen in einer hellen Substanz (wahrscheinlich Kernsaft) eingebettet. Nach den Polen zu konvergieren sie etwas; ihre Enden sind aber nicht mit einander vereinigt, sondern hören frei auf; diejeniger Strahlen um die beiden Central-körperpaare welche direkt auf die Enden der Spindelfasern zu verlaufen, treten mit diesen allem Anschein nach nicht in Kontinuität."

This mutual independence of the spindle-fibers and the astral rays in *Scutigera* is the more clearly shown by the great dis-

similarity in their structure and general appearance; for while the latter are very fine and delicate and do not appear to be separate threads, but resemble rather mere elongations in the network of the cytoplasm, the former are very heavy, stain an intense black, and present the appearance of thick wires, the ends of which may be distinctly seen lying free in the cytoplasm.

At the opening of the metaphase (Fig. 17), the two centrosomes lie at opposite poles of the cell, surrounded by the clear transparent area and the radiating astral rays. The spindle lies directly through the center of the cell, pointing toward the centrosomes but not extending to them. It consists of heavy, intensely staining fibers. At their extremities these converge, but do not unite. In the equatorial plate are the chromosomes, nineteen in number, not lying in a circle on the periphery of the spindle, but apparently scattered more or less irregularly in one plane through the equatorial plate. Division and separation of the chromosomes occur immediately, and the two groups soon come to lie at opposite ends of the spindle fibers.

As has been stated above, after the other chromatin has entirely left the karyosphere, the accessory chromosome separates from the nucleolar portion, which immediately breaks up into numerous small particles. When the nuclear membrane has disappeared, these particles are, of course, cast out into the cytoplasm. They are not dispersed at once throughout the whole cytoplasm, but in the metaphase, take up a fairly definite position at each pole of the spindle proper, midway between centrosome and equatorial plate. Thus, before the attractive force of the centrosomes has succeeded in pulling apart the chromosomes, these particles already lie in the cytoplasm beyond the extremities of the spindle fibers. Here they remain as long as the centrosome is discernible, but after the latter disappears they wander out throughout the cytoplasm and soon degenerate.

Each centrosome, from the time it first becomes visible, consists of two minute granules, surrounded by a transparent space from which the short and delicate astral rays emerge (Figs. 12, 17). But at this period, shortly following the separation of the chromosomes, each centrosome becomes surrounded by a small, spherical-shaped region (Figs. 18, 19), which, when the

cells are stained but lightly by the iron-hæmatoxylin, presents a grayish appearance. In the center of this space, side by side, lie two black granules. When the cell is stained a longer period with the hæmatoxylin, this mass becomes very dark like the centrosomes, and shows an irregular outline, appearing as if formed by the fusing of the converging astral fibers. P. Bouin ('03), in a recent paper under the title of "Centrosome et Centriole" calls this entire structure, consisting of the two granules and the surrounding sphere, the centrosome, and each individual granule, a centriole; using this method to prove that the centrosome is not a permanent organ of the cell. But he seems to have misunderstood the meaning which Boveri, the originator of these terms, applied to them. The centrosome, as described by the latter, is "Ein Körper, an den die Sphärenradien direkt herantreten, ist das Centrosome." He tells us that it is present at all stages of cell-division, and divides to form the centers of the daughter-cells. According to his description this may, in the course of development, enlarge gradually, and become more complex, until just preceding the metaphase it may take on the form of a rather large, well-defined sphere in the center of which one or more minute central granules or centrioles may appear. Later, he adds as a test for distinguishing this enlarged centrosome from the centrosphere, that through the latter *astral rays may be traced*, while the former shows no such differentiation. Bouin accepts Boveri's definition for the enlarged centrosome during the metaphase, but rejects his definition of a centrosome during the prophase and declares the two granules occurring at that time to be the centrioles. In this manner he tries to prove that the centrosome is not a permanent organ of the cell. That his assertion is not valid in the case of *Scutigera* is proved absolutely by the fact that at no time during the existence of the sphere surrounding the two granules does it appear sharply defined from the astral rays, but these seem to traverse it and radiate from the two central granules which are invariably discernible in those cells not too darkly stained.

After the chromosomes have reached their destinations at the extremities of the spindle-fibers (Fig. 19), the asters surrounding the centrosomes fade away and the centrosomes themselves dis-

appear. Either they lose their staining capacity or, having no surrounding rays to mark them, are indistinguishable from the numerous particles, the remains of the nucleolar portion of the karyosphere, massed at this point. The chromosomes, having reached the end of the spindle-fibers at first appear to be attached to them at their extremities (Fig. 19), but as the fibers at once begin to undergo degeneration and to become considerably shortened, the chromosomes soon lie free in the cytoplasm (Fig. 20). While attached to the fibers they lie massed together (Fig. 20), but afterwards they spread over a greater area (Fig. 21) and the separate elements may be seen. Each daughter-cell contains eighteen chromosomes, which are still dumb-bell shaped, but slightly granular and less intensely staining than before. One cell contains in addition the accessory chromosome (Fig. 21), still homogeneous in consistency and staining black. This element, on account of its resemblance to the other chromosomes (during the metaphase) could not be distinguished from them, but at this period may plainly be discerned as it retains the same form and staining capacity possessed throughout the metaphase.

During the period immediately following the reconstruction of the nuclear membrane takes place. This process is the most remarkable phenomenon in the spermatogenesis of *Scutigera*, but as it takes place much more slowly in the second mitosis, affording better occasion for the study of the separate steps, it will be discussed in connection with that division.

The period between the two divisions passes quickly, although the chromosomes undergo great changes in appearance. During the metaphase of the first division, as was stated above, they assume a dumb-bell shape and appear homogeneous (Fig. 17). Immediately after this division they again present the same form and appearance, although reduced in size. As the telophase approaches, they become somewhat more granular, but retain the same general outline. After the reconstruction of the nucleus has taken place, however, the chromosomes gradually become more and more granular until they lie spread through the nuclear area as diffuse masses. Their identity is not lost, although they retain no definite form. As the membrane again breaks down preparatory to the second division, they gradually contract and assume once more their dumb-bell shape.

The formation and appearance of the spindle fibers up to the time of the metaphase has been described above (Fig. 16). During this period they lie like heavy black threads in the transparent substance derived from the nucleus (Fig. 17), one fiber being attached to each chromosome and so distinct that it may be traced from one end of the spindle to the other. The net work throughout the cell, except in the immediate vicinity of the centrosomes, is of the same regular nature characteristic of the cell during the resting stage, and shows the same density marking it throughout the prophase. Around the centrosomes, however, the astral rays are beginning to elongate and strengthen. At the same time the net-work surrounding the spindle becomes more ragged, the cytoplasm through the equatorial plane lighter and freer from linin until by the time the chromosomes have reached their destination at the extremities of the spindle fibers (Fig. 19) the entire area in the vicinity of the spindle is clearer, and scarcely any trace of the net-work can be discerned. Gradually now the spindle fibers spread across the equatorial plane (Fig. 20). As those upon the outside approach the periphery of the cell, where the constriction of the wall has already commenced, they become shorter and weaker as their extremities become dissolved in the cytoplasm through which they are scattered. At no time are the astral rays strong nor do they ever consist of heavy distinct fibers like those of the spindle, but they appear much lighter and at the extremities become lost in the net-work of the cytoplasm. They extend between the centrosomes and the equatorial plate but they never cross in this region. Indeed, scarcely ever can they be distinguished this far, but fade into the network of the cytoplasm about half way from the centrosomes to the equatorial plane (Fig. 17). However, the whole cytoplasm of the cell, extending from the centrosomes at the poles to the constricting cell-wall at the sides, soon becomes transparent and entirely free from net-work, apparently indicating some influence of the centrosomes on this area (Fig. 20).

As the constriction of the cell-wall continues, the regular network of the cytoplasm begins to re-form about the periphery, and the spindle fibers lying in the equatorial region become pushed together until they form a sheaf-shaped bundle, the ends

of which extend far into the cells. The fibers are exceedingly numerous, but not so heavy nor so darkly staining as when attached to the chromosomes during the metaphase and directly following it. Considering the size of the spindle, its disintegration occurs in a remarkably short time, indeed it begins as soon as the chromosomes have reached the extremity of the spindle. Until this time, the fibers were extremely heavy and presented a smooth appearance. But now they gradually become fainter and more granular, while those at the sides appear more and more feeble, until they can scarcely be distinguished from the network of the cytoplasm (Fig. 20). By the time they are pushed in to form the sheaf-like bundle (Fig. 24), the separate threads are of extreme fineness and delicacy. As the cell-wall presses inward, drawing the spindle together, a row of darkly staining bodies forms on the fibers in the equatorial plane, and appears to fuse. Only those fibers on the periphery seem to be affected thus, so that these granules do not form a plate, but a band around the circumference of the spindle. The period between the formation of the sheaf and the disappearance of the fibers passes quickly, although there is a wide variation in the time at which this takes place. In some cases the chromosomes are still in the vesicular stage after the cells have separated and the spindle fibers become greatly reduced in strength and numbers (Fig. 22), whereas in other instances (Fig. 24), a complete reconstruction of the nucleus has taken place before invagination of the cell-wall is completed. However, in no case does the second division commence until the spindle has so completely broken down that no trace of it remains.

During this entire period, the centrosomes are not discernible, but in the next metaphase they have reappeared, one granule on the cell-wall at each pole, surrounded as in previous division by a centrosphere and radiating fibers. These present an appearance almost identical with those in the preceding mitotic figure and bear about the same relative strength to the spindle fibers. As the nuclear membrane breaks down previous to the second mitotic division, the chromosomes all resume the same dumbbell shape as in the telophase of the former division. The accessory chromosome again becomes lost among the others as they

now possess the same form and staining reaction that it has maintained thus far throughout its course.

The metaphase passes rapidly, and soon the chromosomes, as in the preceding division, come to lie at the extremities of the spindle, which also closely resembles that of the first spermatocyte. The chromosomes soon become detached from the ends of these fibers and lie free in the cytoplasm. Generally they are aggregated more or less closely in one region, near where the extremity of the spindle fibers lay, but often they become scattered irregularly through the central area of the cell.

And now an occurrence takes place which has not been previously described in Myriapods, nor am I acquainted with the phenomenon in the male cell of any form, although it is a common occurrence in the egg cell. Exception to this statement should perhaps be made regarding somewhat similar conditions reported by Meves in *Paludina* and *Pygæra*. In this work Meves ('03) reports a vesicular condition of the chromosomes in the telophase of both spermatocyte mitoses, but this may persist after the nuclear membrane is formed. The conditions in *Scutigera* are therefore more comparable to those manifested by various ova. There is no immediate formation of a nuclear-membrane, but each separate chromosome, as it disintegrates, becomes enclosed in a membrane of its own, thus forming a structure similar to a nucleus, but containing only a single chromosome (Fig. 25). The diameter of each of these is no greater than twice that of the chromosome itself. During the formation of these vescicles, the chromosomes become somewhat granular and often lose entirely their typical dumb-bell outline. They assume various arrangements in different cells; sometimes they lie close together, often they are scattered throughout the central region of the cell. Immediately after forming, however, these vesicles begin to fuse with one another, gradually producing large vesicles which contain more chromosomes. There seems to be no definite order of union, for sometimes they join in pairs (Fig. 26) and these gradually come together, while at other times several unite, forming a large vesicle around which are clustered smaller ones (Fig. 27). This latter case seems to occur more frequently although the former is often met with.

As was suggested above, this process occurs in both spermatocyte divisions, but in the first, it takes place much more quickly than in the second, and thus offers fewer examples for study. In case of the first division (Fig. 22), these vesicles always lie close against one another, whereas at the close of the second division, they are often widely scattered through the cytoplasm (Fig. 25). In the first division, too, the nucleus immediately after fusing becomes spherical, and all traces of its vesicular condition are lost. But at the close of the second division, the nucleus, in place of at once becoming spherical, retains for a considerable period traces of its former condition (Fig. 28), and often loses them only when transformation into the spermatozoön takes place. The membrane surrounding the nucleus at first appears very thin, like that of the vesicles; but soon after fusion is completed, it seems to increase considerably in thickness, and resembles that of the first prophase.

The only case which I have found of an occurrence resembling this was reported by Sutton, '00 (*Brachystola magna*). The metaphase is passed without exhibiting any unusual phenonenon. But during the telophase, an occurrence somewhat similar to that found in *Scutigera* takes place. "Each chromosome, on reaching the pole, begins to disintegrate, and at the same time, reconstructs its share of the nuclear membrane as a closed vesicle about itself. Later, all these vesicles become intercommunicating at their polar extremities, with the exception of one, which remains absolutely independent throughout its entire existence. The chromatin of the ordinary chromosome becomes diffused evenly in the nuclear space, while that of the one in the separate vesicle (the accessory chromosome) is deposited upon the inner surface of its capsule."

The spindle of the second mitotic division is markedly different from that of the first, although in the metaphase the two bear a distinct similarity. During the telophase, the breaking down of the net-work of the cell in the equatorial region and the spreading of the spindle fibers across this area is essentially the same as in the previous division, except that the fibers here are considerably fewer. As a result, no prominent sheaf-like bundle is formed when the constricting of the wall occurs, but these

fibers lie like a few strands obliquely through the cytoplasm (Fig. 25, *et seq.*). As soon as the chromosomes reach their destination at the extremity of the spindle, the net-work of the cell commences to re-form, and, within a remarkably short period, all trace of division is lost from the cytoplasm.

Soon after the second division occurs, the centrosomes also disappear. In the case of the former division, it was stated that they either lost their staining capacity or became indistinguishable from the remains of the karyosphere massed in their vicinity. But in this instance, the former is shown conclusively to be the case, partly because of the fact that here there are no such granules with which they could become confused, and partly for the reason that for a considerable time the aster remains visible, although at the point from which the fibers radiate, no centrosome is to be seen (Figs. 25, 26).

I wish to express my gratitude to Dr. C. E. McClung under whose direction the work was done, and to Mr. M. W. Blackman, for assistance in supplying material and for many valuable suggestions.

LABORATORY OF ZOÖLOGY, UNIVERSITY OF KANSAS,
June 8, 1904.

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XPLANATION OF PLATES.

All drawings are from camera-lucida outlines, taken with a B. and L. one-twelfth inch oil immersion objective and one-half, three-fourth, 1 inch B. and L. oculars. In reproduction a reduction of one-sixth has taken place.

EXPLANATION OF PLATE II

FIG. 1. × 2,000. Spermatogonium in late prophase, about the time of the breaking down of the nuclear-membrane. The chromosomes, 37 in number, are spread over the central region of the cell. All are of a slightly granular consistency, except one, the accessory chromosome, which is homogeneous.

FIG. 2. × 1,000. Division of the last spermatogonium. (*a*) Chromosomes arranged on periphery of spindle or (*b*) scattered across equatorial plate. (*c*) Division figures typical. Centrosomes at apices of spindle. (*d* and *c*) Early and late anaphases.

FIG. 3. × 1,000. Late telophase of last spermatogonium. All chromosomes except accessory have become granular and lengthened into threads. The accessory is stiil homogeneous.

FIG. 4. × 1,000. Early spermatocyte. Chromatin segments beginning to form the karyosphere. The remaining segments scattered over nuclear area, in the form of long, granular threads. Growth period commenced.

FIG. 5. × 1,000. Later stage. Karyosphere becoming larger. Remaining segments of chromatin spread over the nuclear area in the form of very diffuse masses.

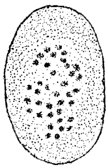
FIG. 6. × 1,000. Chromatin almost all in the karyosphere. Metaplasm bodies spread through the nucleus in considerable quantities. A less amount in the cytoplasm.

FIG. 7. × 1,000. Cell of mature size ready for the prophase. Karyosphere shows no differentiation. Metaplasm bodies decreased in amount. Network in cytoplasm is finer and stains more densely than that of nucleus.

FIG. 8. × 1,500. Karyosphere as seen in various stages. (*a*) During early growth period. (*b*) Just preceding the active prophase. Shows slight differentiation. Several vacuoles present in the matrix of spongy chromatin. (*c*) Karyosphere of same stage, showing the chromatin massed in more definite areas. (*d*, *e*) Very early prophases. Chromatin has become less densely massed; vacuolar substance has increased in amount; and has aggregated into one or several large vacuoles. (*f*) Part of chromatin has left the karyosphere. (*g*) Later stage. But little chromatin remains within the karyosphere, and this is massed about the large central vacuole. (*i*) All chromatin has left the karyosphere except one chromosome, which is probably the accessory. (*j*, *k*) Stages in the disintegration of the karyosphere, after the chromatin has all emerged.

FIG. 9. × 1,500. Early prophase. Chromosomes just commencing to leave the karyosphere. Cloud-like masses of achromatic matter beginning to arise in the nuclear area.

FIG. 10. × 1,500. Later stage in prophase. Chromatin segments granular and in the form of split threads.



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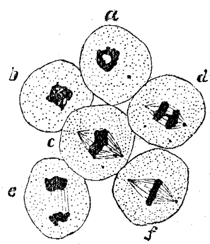

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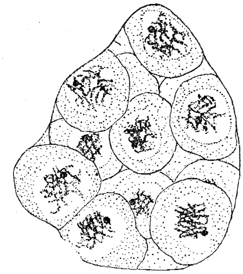

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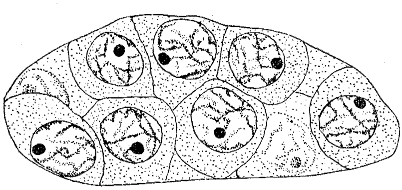

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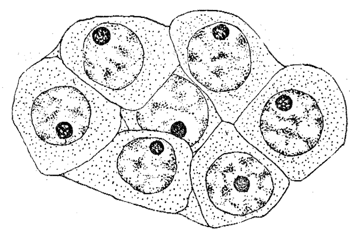

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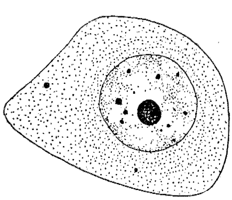

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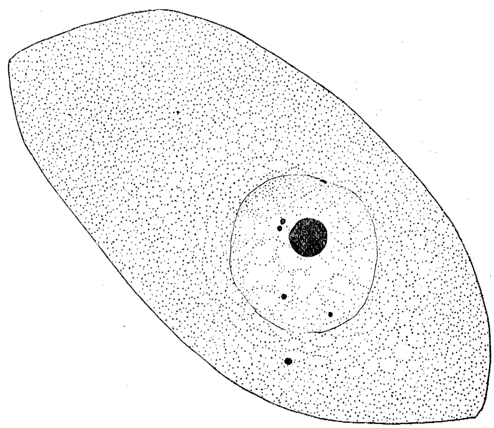

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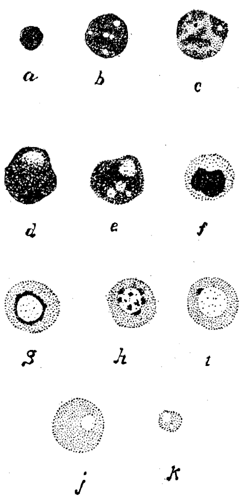

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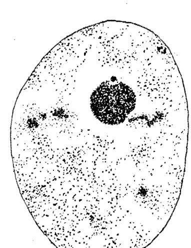

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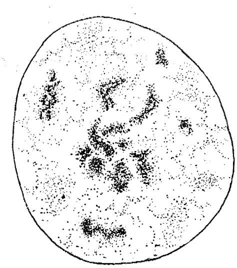

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Explanation of Plate III

Fig. 11. $\times$ 1,500. Later stage. Chromatin segments have broken into a number of small fragments. These are beginning to contract to form tetrads.

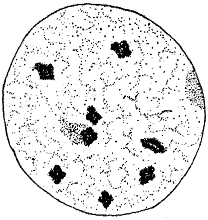
Fig. 12. $\times$ 1,500. Entire cell during mid-prophase. Chromatin in the form of cross-shaped tetrads. Centrosomes in the cytoplasm midway between nuclear membrane and the cell-wall.

Fig. 13. $\times$ 1,500. Later stage. Tetrads more contracted. Linin net work beginning to break down into more definite, less branched fibers.

Fig. 14. $\times$ 1,500. About the same stage. Tetrads in various stages of advancement.

Fig. 15. $\times$ 1,500. Later stage, shortly preceding the breaking down of the nuclear membrane. Chromosomes are nearly all homogeneous, but still preserve their tetrad outline. Nucleolar part of karyosphere still intact.

Fig. 16. $\times$ 1,000. Very late prophase. Nuclear membrane has disappeared and spindle is forming. Chromosomes have changed from tetrad to dumb-bell shape. Nucleolar remains of karyosphere grouped irregularly in the cytoplasm about the centrosomes.



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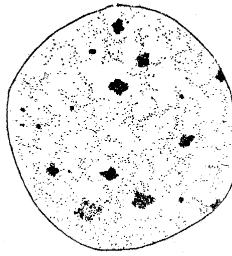

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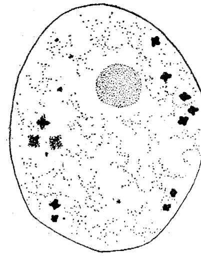

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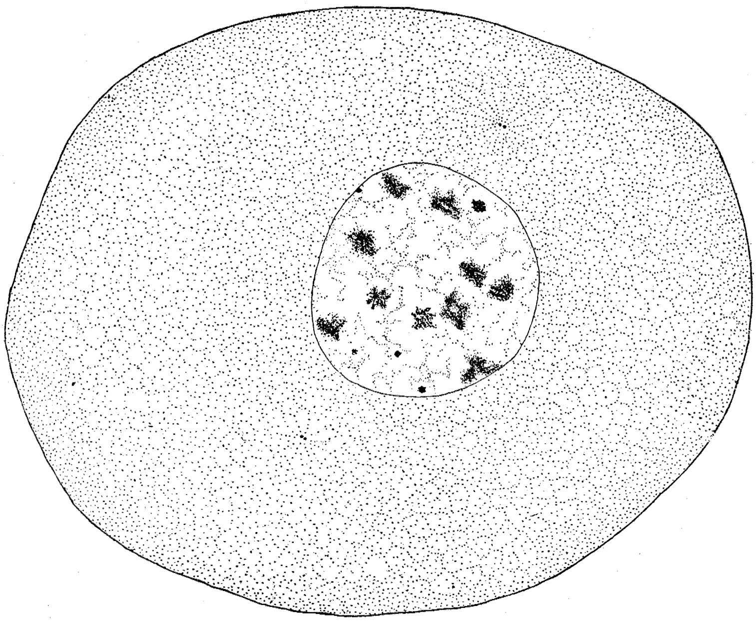

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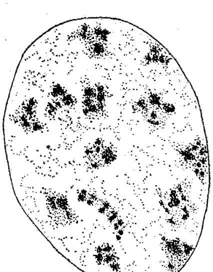

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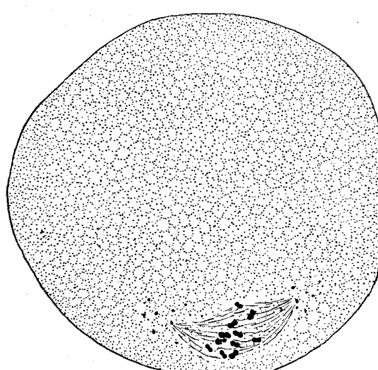

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Explanation of Plate IV

Fig. 17. $\times$ 1,000. Metaphase of first spermatocyte. Chromosomes grouped in equatorial plate. Centrosomes on cell-membrane. Spindle fibers do not extend to centrosomes, but lie free in the cytoplasm, their ends converging slightly. Nucleolar remains of karyosphere drawn to extremities of spindle fibers.

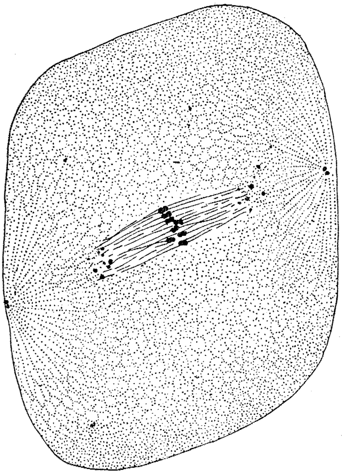
Fig. 18. $\times$ 1,000. Early anaphase. Chromosomes have divided, and again assumed their dumb-bell shape. Nucleolar remains of karyosphere drawn farther toward the centrosomes. Net-work still regular.

Fig. 19. $\times$ 1,000. Late anaphase. Chromosomes at poles of the spindle. Nucleolar remains of karyosphere drawn almost to cell-wall. Net-work beginning to break down in equatorial plate.

Fig. 20. $\times$ 1,000. Early telophase. Chromosomes massed at ends of spindle fibers, which have spread across equatorial region and begun to disintegrate at the extremities. Centrosomes have disappeared. Region from centrosomes to equatorial area considerably lighter, showing influence of centrosomes.

Fig. 21. $\times$ 1,000. Spindle forming into sheaf-shaped bundle. Chromosomes somewhat spread, becoming granular — except accessory, which is still homogeneous.

Fig. 22. $\times$ 1,000. Telophase of first spermatocyte. The vesicles in each one of which a chromosome has become enclosed are beginning to fuse.



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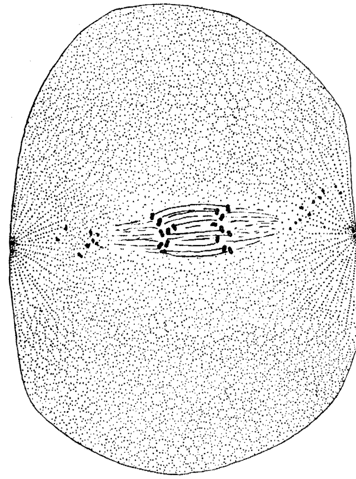

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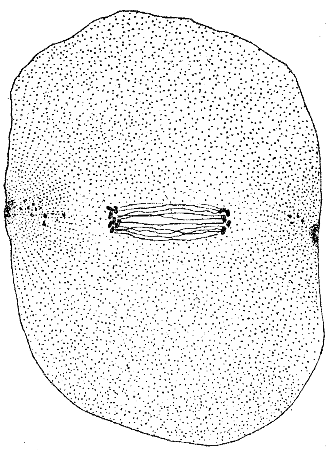

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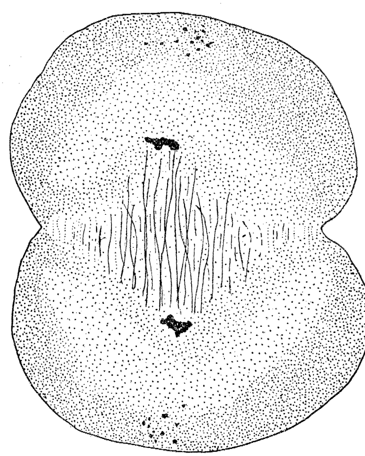

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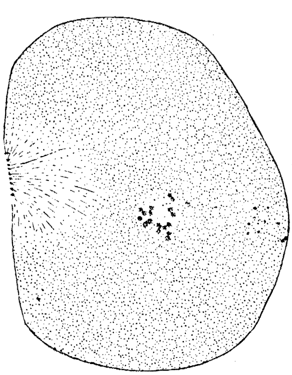

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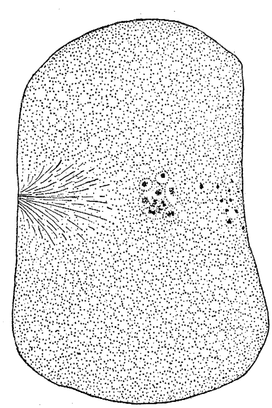

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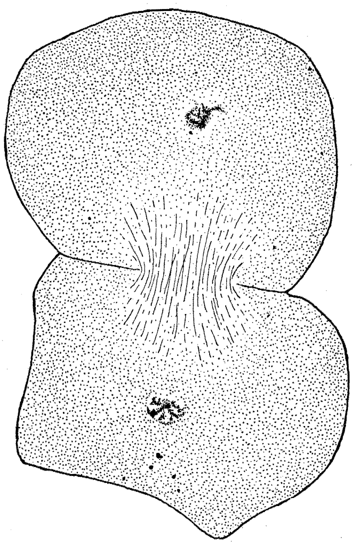
Explanation of Plate V

FIG. 23. $\times$ 1,000. Telophase. Nucleus just forming. Showing the slowness of the forming of the cell-wall in some instances. Compare with Fig. 22.

FIG. 24. $\times$ 1,000. Late telophase. Nuclear membrane formed. Chromosomes become diffused. Cell "A" shows accessory chromosome, which is absent in "B."

FIG. 25. $\times$ 1,000. Telophase of second spermatocyte. Each chromosome is enclosed in its separate vesicle. Centrosome has disappeared, but astral rays are still present. Spindle fibers persist as a few strands lying in the cytoplasm.

FIG. 26. $\times$ 1,000. Slightly later stage. Chromosome vesicles have begun to fuse.



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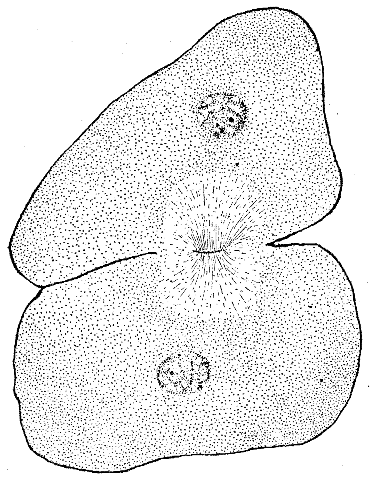

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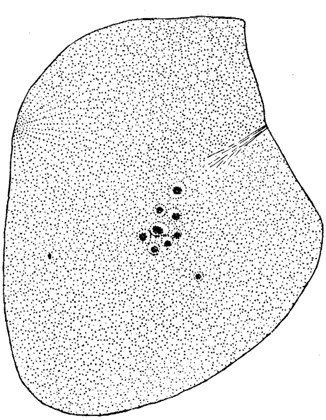

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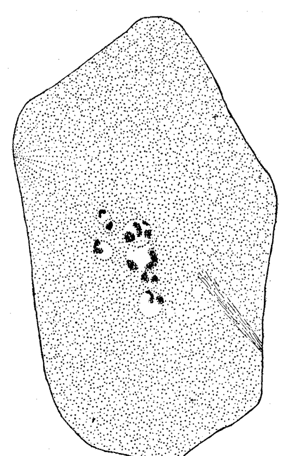

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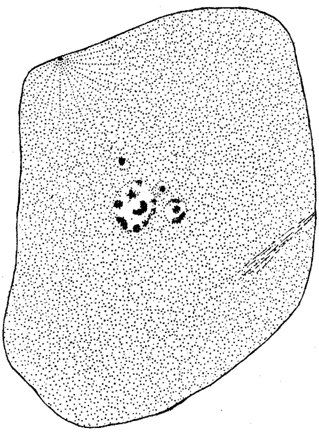
EXPLANATION OF PLATE VI

FIG. 27. × 1,000. Still later stage. Fusion of chromosome-vesicles more advanced. Several of these have united, leaving part of the chromosomes still in separate vesicles.

FIG. 28. × 1,000. Fusion of the chromosome-vesicles complete, but the nucleus still retains a slight trace of its former vesicular condition. Nuclei of this shape aref often to be found until transformation into spermatozoa takes place. All trace o astral rays has disappeared.

FIG. 29. × 1,000. All trace of vesicular stage has disappeared from the nucleus. Typical cell at the close of the second spermatocyte division.



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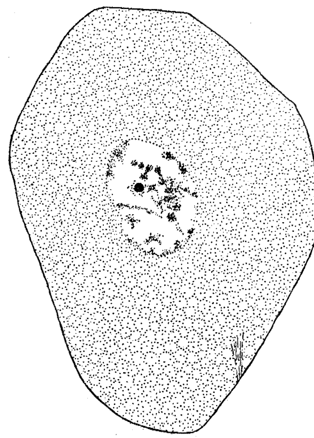

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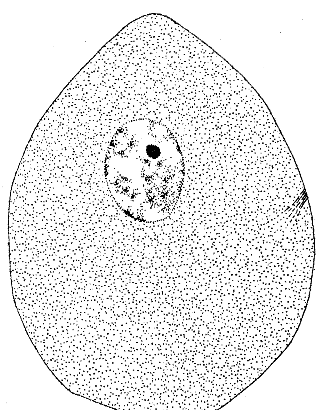

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